

MATRIX-ASSISTED LASER DESORPTION WITH HIGH IONIZATION YIELD

BACKGROUND

[0001] The invention relates to the generation of analyte ions from solid samples on surfaces by matrix-assisted laser desorption (MALDI). One important type of ionization for biomolecules is ionization by matrix-assisted laser desorption (MALDI), which was developed by M. Karas and F. Hillenkamp, in particular, some twenty years ago, and for whose basic research Koichi Tanaka was awarded the 2002 Nobel Prize. MALDI ionizes the biomolecules, which are located in highly diluted form in a mixture with molecules of a matrix substance in samples on sample supports, by bombarding them with pulses of laser light. The ratio of analyte molecules to matrix molecules is, at the most, approximately one thousand to ten thousand, although the analyte substances can form a mixture in which concentration ratios covering several orders of magnitude may pertain between the different analyte substances to be measured.

[0002] MALDI is a competing technique to electrospray ionization (ESI), which ionizes analyte molecules dissolved in a liquid, and can hence be easily coupled to separation techniques such as liquid chromatography or capillary electrophoresis. MALDI has many advantages, however. Hundreds of samples can be applied to a single sample support. Pipetting robots are available for this purpose. It takes only fractions of seconds to transport a neighboring sample with the sample support into the focus of a UV pulsed laser; as much time as is ever needed is then available for the analysis of this sample, the only limit being when the sample is completely exhausted. This sets MALDI very favorably apart from electrospray ionization, which provides only a very slow sample change and, when used in conjunction with chromatography, necessarily limits the analysis time to the duration of the chromatographic peak. MALDI is, for example, ideal for the identification of tryptically digested proteins which have been separated by 2D gel electrophoresis and whose separated fractions have been processed into separate MALDI samples. MALDI analysis of peptides separated by liquid chromatography and applied to MALDI sample supports is also gaining ground ("HPLC MALDI"). Of particular interest is the use of MALDI in the imaging mass spectrometry of histologic thin sections, which can determine the spatial distribution of individual proteins and also of individual pharmaceuticals or their metabolites.

[0003] The lasers usually used for MALDI are UV lasers providing pulses of laser light beams of a few nanoseconds duration, focused by lenses onto focal spots of between approximately 100 and 200 micrometers diameter. The focusing adjustment is deliberately chosen to give these diameters; the "focal spot" on the sample does not correspond to the achievable minimum focal diameter of the beam of laser light. The ions of every single pulse of laser light are accelerated axially into a time-of-flight path in specially designed MALDI time-of-flight mass spectrometers; after passing through the flight path, the ions are fed to a detector, which measures the mass-dependent arrival time of the ions and their quantity, and then records the digitized measured values in the form of a time-of-flight spectrum. The laser light pulses used here have repetition rates of up to 2 kilohertz approximately. The measured values of a few hundred sequentially obtained time-of-flight spectra of the ions from the individual pulses of laser light are added together to form a sum spec-

trum: this is subjected to a peak separation procedure, and the list of the time-of-flight peaks is converted into a list of masses and their intensities using a calibration curve. This list is called a "mass spectrum".

[0004] One disadvantage of this usual MALDI method, however, is that it ionizes only around one ten thousandth of the analyte molecules. Only 60 or so analyte ions are obtained from one attomol of an analyte substance, i.e. from approx. 600,000 molecules. The rest are not ionized; an unknown proportion of the remaining molecules are possibly contained in ejected lumps or molten splashes of matrix substance and are completely excluded from ionization, while, on the other hand, an also unknown proportion of the analyte molecules are simply not ionized in the laser desorption process.

[0005] Matrix-assisted laser desorption has, until now, mainly been performed in a high vacuum with direct axial injection of the ions into the flight path of a specially designed MALDI time-of-flight mass spectrometer. The starting point (with few exceptions) is solid sample preparations on a sample support. The samples consist primarily of small crystals of the matrix substance, to which a small proportion (only around one hundredth of one percent at most) of molecules of the analyte substances are added. The "analyte substances" themselves can consist of a mixture of diverse analyte substances. The analyte molecules are embedded individually into the crystal lattice of the matrix crystals, or are located in crystal boundary surfaces. The samples prepared in this way are irradiated with short pulses of UV laser light. The duration of the pulses is usually between three and ten nanoseconds. This produces vaporization clouds which contain ions of the matrix substance as well as some analyte ions. Some of the analyte ions are already contained in the solid sample in ionized form; some are created directly in the explosive vaporization process in the hot plasma; and a third fraction is formed in the expanding cloud by proton transfer in reactions with the matrix substance ions.

[0006] The very detailed review article "The Desorption Process in MALDI" by Klaus Dreisewerd (Chem. Rev. 2003, 103, 395-425) reports on the influences of many parameters, such as spot diameter, laser light pulse duration and energy density, on the desorption and the generation of the matrix ions and analyte ions. Although the influences of many of these parameters are not independent of each other, the step of carefully varying all the parameters in relation to each other has been almost entirely neglected. It has been reported, for example, that the laser light pulse duration of between 0.55 and 3.0 nanoseconds has no influence on ion formation; but the spot diameter here was neither varied nor even stated. On the other hand, the energy density threshold for the initial occurrence of ions has been investigated for varying spot diameters without, however, investigating the profile of the energy density in the laser spot, which, according to our own investigations, is of immense importance. According to this literature source, incidentally, this threshold increases very strongly with decreasing spot diameters: for spot diameters of approx. 10 micrometers, around ten times the energy density (fluence) is required compared to spot diameters of 200 micrometers. We cannot confirm this. Apparently, nothing is elucidated in the literature on the mutual influence of spot diameter and laser pulse duration.

[0007] Previous investigations into the MALDI process were, however, adversely affected by un-reproducible sample preparation methods. Usually, droplets with matrix and analyte solution have simply been applied to the sample support